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Targeting the ACE2-Ang-(1–7) Pathway in Cardiac Fibroblasts to Treat Cardiac Remodeling and Heart Failure

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Abstract

Fibroblasts play a pivotal role in cardiac remodeling and the development of heart failure through the deposition of extra-cellular matrix (ECM) proteins and also by affecting cardiomyocyte growth and function. The renin-angiotensin system (RAS) is a key regulator of the cardiovascular system in health and disease and many of its effects involve cardiac fibroblasts. Levels of angiotensin II (Ang II), the main effector molecule of the RAS, are elevated in the failing heart and there is a substantial body of evidence indicating that this peptide contributes to changes in cardiac structure and function which ultimately lead to progressive worsening in heart failure. A pathway involving angiotensin converting enzyme 2 (ACE2) has the capacity to break down Ang II while generating angiotensin-(1–7) (Ang-(1–7)), a heptapeptide, which in contrast to Ang II, has cardioprotective and anti-remodeling effects. Many Ang-(1–7) actions involve cardiac fibroblasts and there is information indicating that it reduces collagen production and also may protect against cardiac hypertrophy. This report describes the effects of ACE2 and Ang-(1–7) that appear to be relevant in cardiac remodeling and heart failure and explores potential therapeutic strategies designed to increase ACE2 activity and Ang-(1–7) levels to treat these conditions.

1. Description

a. Cardiac fibroblasts and remodeling

Despite important advances in the treatment of heart failure over the past several decades, morbidity and mortality in patients with this condition remain at unacceptably high levels [1,2]. Remodeling of the heart which occurs in response to injury and/or an increase in wall stress plays a key role in the progressive deterioration of cardiac function that leads to heart failure [3,4]. Thus, new therapies that can inhibit remodeling would be expected to improve outcomes. Remodeling is characterized by cardiac hypertrophy and dilatation as well as conformational changes in the shape of the heart. Cardiac fibrosis which develops at sites distant from local injury (e.g. in segments of non-infarcted myocardium in patients with ischemic cardiomyopathy) occurs during remodeling [5,6] and this process adversely affects systolic and diastolic functions of the heart and promotes cardiac arrhythmias. Deposition of extra-cellular matrix (ECM) depends primarily on cardiac fibroblasts which produce many

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of the interstitial proteins in the heart and release enzymes such as matrix metalloproteinases and tissue inhibitors of metalloproteinases which further regulate the balance between ECM production and breakdown [7–11]. There is also evidence that cardiac fibroblasts release growth factors that act in a paracrine manner to stimulate cardiomyocyte hypertrophy [12–15].

b. Role of the renin angiotensin system (RAS) in remodeling

In addition to helping maintain cardiovascular homeostasis through its influence on salt and water regulation and vascular tone, the RAS plays an important role in maladaptive cardiac remodeling [5,16]. The mechanisms through which the RAS contributes to the remodeling process include indirect effects leading to increased load on the heart, interactions with other neurohormonal systems and by direct effects on cardiac cells. Angiotensin II (Ang II), the main effector molecule of the RAS, acts predominantly through its Type 1 receptor (AT₁) to activate cardiac fibroblast functions that increase the amount of ECM in the heart [17,18]. Although Ang II has been shown to stimulate cardiomyocyte hypertrophy, this activity appears to be mainly indirect since application of the peptide to cultured cardiomyocytes has little effect on cell growth or synthesis of proteins [15]. Culture media taken from adult cardiac fibroblasts, however, can stimulate cardiomyocyte hypertrophy and this effect is significantly enhanced by Ang II activation of cardiac fibroblast AT₁ receptors [15]. These observations are consistent with evidence that the AT₁ receptor density is substantially greater on cardiac fibroblasts than on cardiomyocytes [17]. Moreover, the increase in AT₁ receptors that occurs in the heart as it remodels involves predominantly non-myocytes [19,20]. Thus, the cardiac fibroblasts appear to be the predominant target for the RAS during cardiac remodeling.

c. Alternative pathways of the RAS

The local tissue-based cardiac RAS is regulated independently of the systemic circulatory RAS and its activation is central to the remodeling process [16]. Cells within the heart generate all components of the RAS (or, in the case of renin activity, extract it from the coronary circulation [21]) and both Ang II levels and AT₁ receptor density are increased in the myocardium of remodeling hearts [5,19,20]. Moreover, results from studies performed in experimental animal models and from clinical trials in human patients provide unequivocal evidence that strategies designed to block RAS activation favorably influence cardiac remodeling [22–24] and improve outcomes including increased survival [22,25–27].

While Ang II through its AT₁ receptor is involved in most physiologic and pathophysiologic effects of the RAS, additional pathways within this system that are relevant in health and disease are known to exist. A homologue of angiotensin converting enzyme (ACE) termed angiotensin converting enzyme 2 (ACE2) was reported independently by two groups in 2000 [28,29]. ACE2 has approximately 40% amino acid sequence similarity with ACE and both enzymes exist as Type I integral membrane-bound glycoproteins. The 805 amino acids of ACE2 include an amino-terminal signal peptide, a catalytic ectodomain, a transmembrane domain and a carboxyl-terminal cytoplasmic domain. The single metalloprotease zinc binding motif is located in the ectodomain and ACE2 functions as a carboxymonopeptidase. As shown in the Figure it catalyzes the conversion of Ang I to angiotensin-(1–9) which, in turn, can be converted to Ang-(1–7) by other peptidases, and hydrolyzes Ang II to Ang-(1–7) with high catalytic affinity [30]. Thus, whereas ACE generates Ang II, ACE2 reduces Ang II levels while producing Ang-(1–7). Other known substrates of ACE2 include physiologically active peptides such as des-Arg⁹-bradykinin, apelin-13, dynorphin A (1–13), and β -casomorphin [30].

Cleavage of the carboxyl-terminal amino acid from Ang II results in the formation of Ang-(1–7), a heptapeptide with vasodilatory and cardioprotective properties that has also been reported to counteract effects of Ang II that promote adverse cardiac remodeling (Figure). To assess the potential role of Ang-(1–7) on cardiac remodeling we measured the effects of this peptide on selected functions of cultured adult rat cardiac fibroblasts (ARCFs) [15]. Pretreatment of ARCFs with Ang-(1–7) inhibited Ang II stimulated [³H]proline incorporation, suggesting that Ang-(1–7) might reduce fibrous tissue deposition, particularly in situations where Ang II stimulation is involved. Stimulation with Ang-(1–7) by itself also reduced [³H]proline incorporation compared to unstimulated cells. Pretreatment of ARCFs with Ang-(1–7) significantly reduced Ang II stimulated increases in endothelin-1 and leukemia inhibitory factor mRNA, suggesting that the peptide might inhibit synthesis of autocrine/paracrine growth factors by cardiac fibroblasts. To further explore this possibility, cardiomyocytes were exposed to Ang II, Ang-(1–7) or Ang II after Ang-(1–7) pre-treatment. While the angiotensin peptides alone did not induce cardiomyocyte hypertrophy as assessed by [³H]leucine incorporation and atrial natriuretic peptide synthesis, exposure of the cardiomyocytes to ARCF culture media induced a hypertrophic response (compared to mock culture media that had not been in contact with the fibroblasts), which increased significantly when the ARCFs had been stimulated with Ang II. This increase, however, was inhibited by pre-treating ARCFs with Ang-(1–7). Overall, these findings suggest that Ang-(1–7) may be playing an important counter-regulatory role during cardiac remodeling by inhibiting cardiac fibroblast ECM synthesis and release of hypertrophic growth factors.

The pathways through which Ang-(1–7) initiates potentially favorable anti-remodeling effects have not been fully elucidated. While some investigators report that the peptide can act through well-characterized angiotensin receptors such as AT₁ or AT₂ [31–33], there is also evidence that Ang-(1–7) frequently acts via its own dedicated receptor, which is widely regarded to be the Mas receptor [34]. Mas is a G protein-coupled receptor [35] and, although Mas is expressed in cardiac fibroblasts (data not shown), the mechanism by which Ang-(1–7) exerts its effects on these cells is currently unknown.

2. Expression and regulation in animal models

While ACE2 is ubiquitously expressed in multiple organs in both rodents and humans, its expression is particularly high in human heart, kidney and testis [28,29,36]. In the heart ACE2 has been found to be localized to endothelial cells, smooth muscle cells, cardiomyocytes, macrophages [29,37] and myofibroblasts [38]. There is evidence that cardiac ACE2 expression and activity can be regulated at both pre- and post-translational levels. Burell *et al* reported increased cardiac ACE2 mRNA expression and activities in the infarct zone and the ischemic zone surrounding a myocardial infarction (MI) [37], while Ishiyama *et al* showed no change in ACE2 mRNA level at 4 weeks post-MI in rats [39]. Down-regulation of cardiac ACE2 activity and mRNA levels have been described in experimental heart failure in rats at 8 weeks post-MI [40] and decreased protein level was measured after 2–4 weeks of aortocaval fistula [41]. The inconsistency of results in these models of experimental heart failure suggest that regulation of cardiac ACE2 expression and activities varies depending on disease state and time point at which measurements are obtained.

In addition to transcriptional and translational influences, ACE2 also undergoes post-translational modification, known as ectodomain shedding, in which proteases (often referred to as “sheddas”) release the ACE2 ectodomain as soluble forms *in vitro* [42,43] and *in vivo* [44]. Results from our laboratory indicate that this process results in the formation of 2 major cleavage products [43], both of which retain roughly similar enzymatic activities. A Disintegrin and Metalloproteinase 17 (ADAM 17) has been shown to play a

major role in this process, although our recent findings indicate that other sheddases may be involved.

During rat development Mas becomes detectable after birth, increases in expression thereafter and then plateaus at 4–6 months of age [45]. Mas exhibits a fairly broad tissue expression profile, including the heart, kidney, testis, brain, retinal pigment epithelium [46,47], skeletal muscle, liver and adipose tissue [48]. Within the heart, Mas is localized to the vasculature [49], cardiomyocytes [50,51] and fibroblasts (detected by qRT-PCR, data not shown). Although fibroblasts are not the only cells in the heart that respond to Ang-(1–7), our results with cultured cells described above and evidence of the Mas receptor on ARCFs suggest that these cells can be an important target for strategies designed to enhance Ang-(1–7) and Mas effects.

3. Evidence from gain and loss of function

Distinct substrate specificity of ACE and ACE2 suggests different physiological roles of these enzymes. The possibility that ACE2 could be involved in the regulation of the cardiovascular system has been investigated using genetically engineered mice and gene transfer. Crackower *et al* reported impaired cardiac contractility with an increase in local cardiac Ang II level in ACE2 knockout mice and this cardiac phenotype could be rescued by concomitant deletion of the ACE gene [52]. Although other groups have failed to replicate this cardiac phenotype, there are reports in ACE2 null mice of both an increase in blood pressure with altered response to Ang II [53] and reduced cardiac contractility with an increase in cardiac Ang II levels following transverse aortic constriction [54]. Despite these inconsistencies, the totality of evidence indicates a protective role of ACE2 in the cardiovascular system and suggests that the balance between ACE and ACE2 may regulate Ang II levels and effects in some pathologic settings. Evidence that gene transfer of ACE2 into the heart inhibits cardiac remodeling stimulated by Ang II infusion [55], hypertension [56] or MI [57] in rats is consistent with this possibility. Continuous administration of C16, a specific ACE2 inhibitor, to rats beginning 2 days following coronary artery ligation increased infarct size and reduced LV fractional shortening in association with a reduction in the number of c-kit⁺ stem cells that were detected in the peri-infarction zone [58]. Reports of ACE2 overexpression in the heart causing cardiac conduction disturbances in transgenic mice [59] and fibrosis in spontaneously hypertensive rats [60], however, indicate that functions of this enzyme may be more complex than initially believed.

Although administration of an ACE inhibitor [40,61] or angiotensin receptor blocker (ARB) [39,41,61] has been reported to increase cardiac ACE2 mRNA, protein and activity both under normal conditions and post-MI in rats, one study failed to demonstrate significant effects of ACE inhibition on ACE2 [37]. There is evidence that aldosterone antagonists also increase ACE2 protein level and activity in rat cardiac tissue [41] and up-regulate mRNA in macrophages from heart failure patients [62]. These results raise the possibility that at least some of the beneficial effects reported with the use of drugs that inhibit Ang II generation and/or cellular effects could be related to increased expression and activity of ACE2.

Mas overexpression exhibits constitutive activity that includes activation of Rac [63] and G_q/G₁₁ [64]. When expressed at physiologic levels Mas requires stimulation by Ang-(1–7) or to a lesser extent by other angiotensin peptides [65]. Stimulation of Mas has been shown to induce the release of arachidonic acid [34] and nitric oxide [50,66,67] and to upregulate ACE2 expression [68]. The latter suggests a positive feedback mechanism as part of this alternative pathway of the RAS. The interaction of Ang-(1–7) with Mas is also capable of altering other signaling pathways, including attenuation of those activated by Ang II [15,35], glucose [69] and serum [51] and augmentation of those activated by bradykinin [70].

Overall, receptor interactions and cellular effects of Ang-(1–7) appear to be dependent on both cell-type and relative expression level of all of the receptors and signaling intermediates involved.

4. Human data

Increases in cardiac ACE2 expression, protein levels and activity have been reported in patients with heart failure of both ischemic and non-ischemic etiology [37,71,72]. Hydrolysis of Ang II by ACE2 is considered to be a key pathway to generate Ang-(1–7) in the failing heart [71]. Soluble ACE2 activity is increased in the blood of heart failure patients and the level correlates with disease severity [44]. An endogenous inhibitor for ACE2 [73] and inhibitory autoantibodies against ACE2 [74] have been reported to exist in plasma of healthy subjects and patients with inflammatory vasculopathies, respectively.

5. Translation potential, pros and cons

Given that ACE and ACE2 have distinct (even opposing) effects on metabolism of RAS effector peptides that regulate cardiovascular structure and function, drugs which modify the balance of expressions and activities of these enzymes can be viewed as potential therapeutic tools to treat a variety of cardiovascular diseases (Table). As already described, ACE inhibitors, ARBs and aldosterone antagonists have been reported to increase ACE2 activity. This mechanism might contribute to their beneficial effects on cardiac remodeling and thus provide an additional rationale for their use in heart failure patients. From this perspective, the ARBs would appear to have an advantage since they tend to increase Ang II levels, an effect that along with the increase in ACE2 activity would tend to increase the levels of the protective peptide Ang-(1–7). However, superiority of the ARBs to ACE inhibitors in either MI survivors or heart failure patients has not been demonstrated. Recently, Hernandez-Prada *et al* used a conformational-based rational drug strategy to identify small molecules that specifically enhance ACE2 activities [75]. One of these ACE2 activators, Xanthenone, has been demonstrated to decrease blood pressure and improve cardiac function with inhibition of cardiac and renal fibrosis in spontaneously hypertensive rats. Soluble recombinant ACE2 (srACE2) derived from the entire ectoderm was also shown to have inhibitory effects on Ang II-induced hypertension in mice [76]. Thus, agents, in addition to ACE inhibitors, ARBs, and aldosterone antagonists, which can increase ACE2 expression and ACE2 activators or analogs could be novel therapeutic tools to treat cardiovascular diseases, particularly those in which Ang II is involved. Careful attention may, however, be necessary to optimize the potential therapeutic effects of ACE2 since an endogenous inhibitor [73] and autoantibodies against ACE2 [74] have been detected in human plasma, as described above. Administration of srACE2 has been reported to result in the generation of antibodies against ACE2 [77] emphasizing the fact that therapeutic strategies based on the use of srACE2 should be designed to avoid enzyme inhibition resulting from the generation of ACE2 antibodies.

One additional potential strategy would be to regulate the rate of cleavage of ACE2 from the surface of cardiac cells. Retention of enzymatic activity would be anticipated to reduce Ang II and increase Ang-(1–7) within microenvironments of the heart. Such strategies would need to account for the fact that ACE2 is cleaved at more than a single site and that multiple enzymes appear to serve as sheddases in this process. The central role of ADAM17, however, suggests that agents that block its activity could be effective ways of enhancing ACE2 activity in the heart.

Although Ang-(1–7) has been shown to have favorable effects in several rat models of cardiovascular disease including attenuating the development of heart failure post-MI [78–

80], this peptide has limited pharmacologic applications due to its rapid turnover. More stable Ang-(1–7) analogues or Mas receptor agonists could, however, have great potential as cardiovascular therapeutic agents. The nonpeptide Mas agonist, AVE 0991, improves cardiac function in rats with diabetes [80,81], with isoproterenol treatment [82] and following experimentally-induced MI [83]. AVE 0991 has also been shown to ameliorate progression of atherosclerosis in apoE null mice [84]. Another Mas agonist peptide, CGEN-856S, has been reported to be more stable than Ang-(1–7), but it has not been extensively studied [85]. Both agonists, however, have potential drawbacks. Since AVE 0991 crosses the blood-brain barrier it could induce counter-regulatory central effects, while CGEN-856S may not precisely mimic the action of Ang-(1–7) since it induces Ca^{2+} -dependent nitric oxide release, while Ang-(1–7) is reportedly Ca^{2+} -independent [15,85]. To our knowledge no human data exists regarding these Mas agonists, but their clinical potential (as well as that of other future compounds) as cardioprotective agents is recognized. Based on these considerations, further exploration of the effects of stable Ang-(1–7) analogues or Mas receptor agonists in cardiovascular disease is clearly warranted.

6. Most important questions and problems for future research

While evidence is accumulating that targeting ACE2, Ang-(1–7) or the Mas receptor might have therapeutic potential, the effects of enhancing this alternative pathway of the RAS appear to depend on a variety of factors including the approach taken, the specific condition and timing in the progression of the disease state. Strategies that enhance ACE2 activity may prove to be more difficult to develop than those that block its effects. Also, the paucity of information about the pathways involved in Mas signaling, interactions with other receptors and in mediating Ang-(1–7) effects leaves a great deal of uncertainty about the outcomes of strategies designed to target this receptor. Most importantly, however, is the fact that the effects of newly developed reagents directly targeting ACE2 or Mas receptor, such as ACE2 activators, soluble ACE2 and Mas agonists (Table), must be compared with drugs currently used to treat heart failure patients (i.e. ARBs, ACE inhibitors and aldosterone antagonists) to determine if targeting the ACE2-Ang-(1–7) pathway alone or targeting multiple components of the RAS offers any advantages beyond those of existing therapies. As unexpected effects have resulted from modifying the ACE2/Ang-(1–7) pathway [59,60], the potential of each new therapy will also have to be carefully evaluated to be certain that it does not have adverse side effects.

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Figure.

Classical and alternative pathways of the renin angiotensin system. Angiotensin II is the preferred substrate of ACE2 which cleaves the C-terminal amino acid to form a heptapeptide, Ang-(1-7). The relative balance between these peptides could influence the rate of progression of cardiac remodeling and the development of heart failure.

Table

Potential pharmacological therapeutic agents as modulators of expression or functions of ACE2 and Mas receptor.

Target	Reagents	Effects on cardiac ACE2 or mechanisms
ACE2	ACE inhibitors	mRNA ↑ [61] and activity ↑ [40]
	ARBs	mRNA ↑ [39,61] protein ↑ [41] activity ↑ [41,61]
	Aldosterone antagonists	Protein ↑ [41] activity ↑ [62]
	Xanthenone	Synthetic ACE2 activator [75]
	Recombinant ACE2	Soluble active ACE2 [76]
	ADAM 17 inhibitor?	Regulation of ectodomain shedding
Mas receptor	AVE 0991	Nonpeptide Mas agonist [80,81]
	CGEN-856S	Mas agonist peptide [85]
	MBP7	Mas “surrogate peptide ligand” [86]